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1-*O*-β-D-GLUCOPYRANOSYLEUCOMMIOL, AN IRIDOID GLUCOSIDE FROM *AUCUBA JAPONICA*

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Abstract—Eucommiol and a new eucommiol glucoside (eucommioside II) were isolated from *Aucuba japonica*, besides known aucubin The structure of eucommioside II was determined, by ^{1}H and ^{13}C NMR spectroscopy and some chemical transformations, as 1-*O*-β-D-glucopyranosyl eucommiol Similarities between components of *A japonica* and *Eucommia ulmoides* (aucubin, eucommiol, eucommioside I) are discussed briefly

INTRODUCTION

In previous papers, we reported the isolation of eucommiol (**1**) [1] and eucommioside I† (2″-*O*-β-D-glucopyranosyl eucommiol) (**2**) [2], besides large amounts of aucubin (**3**), from leaves and branches of *Eucommia ulmoides* collected in autumn In the same papers [1, 2], we pointed out the similarities in structure and chirality existing between aucubigenin (**4**) (whose glucoside, **3**, is present in large amounts in the plant throughout the year) and eucommiol (**1**), a minor component present only in the autumn as a probable degradation product of **3**

In our search for large amounts of easily available iridoid glucosides as starting material for syntheses in the prostaglandin field, we isolated in autumn from *Aucuba japonica* Thumb aucubin (**3**), as the main component (> 3% of the fr wt of plant), eucommiol (**1**) and a new polar compound **5** (R_f 0 17) which gave the same olive-brown colour as **1** (R_f 0 51) and **2** (R_f 0 17) on treatment with the iridoid reagent (vanillin) This paper deals with the isolation, structure and configuration of compound **5** which we have named eucommioside II because of its isomeric relationship with eucommioside I (**2**)

RESULTS AND DISCUSSION

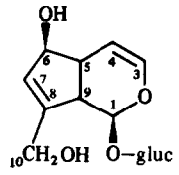
Compound **5** is a water soluble, viscous colourless oil which gives a neutral reaction, and is chromatographically (paper and TLC) indistinguishable from **2** although a slight separation is seen on TLC (see Experimental) with the corresponding peracetates (**6** and **7**)

The UV and IR spectra of **5** are very similar to those of **1** and **2** [1, 2] and show absorptions typical of an isolated C=C double bond at λ_{max} 208 nm ($\log\varepsilon = 3\,6$) and at 1650 cm^{-1} (weak) respectively Enzymatic (β-glucosidase) and acid hydrolysis of **5** affords only D-glucose (1 mol) and a stable aglycone, whose physical and spectral data are identical with those of **1**

Eucommioside II (**5**) is therefore a β-D-monoglucoside of **1** as confirmed by the presence in its ^{1}H NMR spectrum (see Experimental) of a doublet at δ 4 55 (1H, $J_{1',2'}$ = 7 5 Hz) due to the anomeric proton H-1′ in the β configuration As regards the signals from the aglycone moiety, the ^{1}H NMR spectra of **1** and **5** are very similar except for the broad signal of H-1 which overlaps the C<u>H</u>$_2$OH allylic signal at δ 4 24 in **1** and in **5** is slightly deshielded (+ 0 16 ppm) An identical Δδ value is observed for the H-1 signals of the couple **2**–**5** while the shift changes of the 2H-2″ signal in the couple **1**–**2** cannot be measured exactly owing to their overlapping in **2** with D-glucose signals This small deshielding (+ 0 16 ppm) fits very well with the values range (0 15–0 25 ppm), inferred from studies with many other iridoid glucosides [2–4], of the 'O-glucosidation effect' on geminal protons, and therefore shows **5** to be 1-*O*-β-D-glucopyranosyl eucommiol In addition, the linkage of the D-glucopyranosyl unit to the β-hydroxyl group at C-1 causes an interesting, stereodifferentiated effect on the chemical shifts of vicinal protons at C-2 and C-5 In fact, both β-oriented protons at these positions undergo in **5**, with respect to **1**, an appreciable downfield shift of + 0 28 and + 0 18 ppm respectively, while the αH-5 proton, in opposite configuration to that of the β-*O*-D-glucopyranosyl unit, is practically unaffected (see Experimental)

Much more spectroscopic evidence is obtained from the ^{13}C NMR spectra (Table 1) of **5** The PND spectrum is made up of fifteen distinct lines, six of them attributable to the β-D-glucose unit and the remaining nine to the aglycone carbons The chemical shift values of the latter group of signals are nearly identical with those of **1** except for those carbons which are influenced by glucosidation effects, i e C-1 which in **5** is deshielded (α effect) of 8 22 (75 30 → 83 52 ppm), C-2 and C-5 whose resonances in **5** are shielded (β effect) of 1 97 (52 93 → 50 96 ppm) and 2 05 (42 19 → 40 14 ppm) respectively Similar glucosidation α and β effects come out from the comparison of **5** with its positional isomer **2** (C-1, Δδ + 8 34 ppm, C-2, Δδ

†As a result of the isolation of a second eucommiol glucoside, we propose to modify in this way the original name 'eucommioside' [2] By analogy with previous papers on cyclopentenpolıols [1, 2, 9] they are numbered according to IUPAC rules



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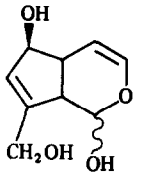

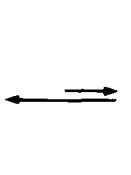

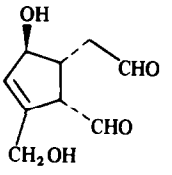

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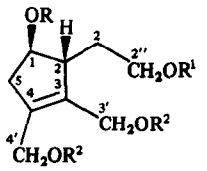

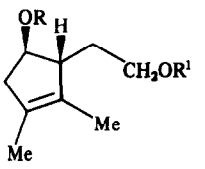

1 R = R¹ = R² = H
2 R = R² = H, R¹ = β–D–gluc
5 R¹ = R² = H, R = β–D–gluc
6 R¹ = R² = Ac, R = β–D–gluc (OAc)₄
7 R = R² = H, R¹ = β–D–gluc (OAc)₄

8 R¹ = H, R = β–D–gluc
9 R = R¹ = OH

Table 1 ^{13}C NMR chemical shift assignments for compounds **1**, **2** and **5–9** The spectra were recorded at 20 MHz

C	**1** (D_2O)	**2** (D_2O)	**5** (D_2O)	**6*** ($CDCl_3$)	**7*** ($CDCl_3$)	**8** (D_2O)	**9** (D_2O)
1	75 30 *d*	75 18 *d*	83 52 *d*	82 12 *d*	76 75 *d*	84 02 *d*	75 62 *d*
2	52 93 *d*	52 95 *d*	50 96 *d*	50 97 *d*	50 77 *d*	53 93 *d*	56 04 *d*
3	137 11 *s*	137 31 *s*	137 02 *s*	134 45 *s*	134 56 *s*	130 10 *s*	130 03 *s*
4	138 96 *s*	138 85 *s*	139 16 *s*	136 04 *s*	136 12 *s*	132 84 *s*	132 62 *s*
5	42 19 *t*	42 20 *t*	40 14 *t*	40 03 *t*	40 33 *t*	43 59 *t*	45 75 *t*
2′	33 11 *t*	30 67 *t*	33 02 *t*	29 58 *t*	30 48 *t*	33 23 *t*	33 29 *t*
2″	60 84 *t*	69 45 *t*	60 67 *t*	62 29 *t*	67 39 *t*	60 84 *t*	61 06 *t*
3′	56 17 *t*	56 25 *t*	56 16 *t*	58 29 *t*	58 50 *t*	11 95 *q*	12 09 *q*
4′	57 91 *t*	57 93 *t*	57 83 *t*	60 04 *t*	60 04 *t*	13 71 *q*	13 77 *q*
1′		103 14 *d*	102 25 *d*	99 88 *d*	100 97 *d*	102 19 *d*	
2′		74 03 *d*	73 94 *d*	71 47 *d*	71 48 *d*	73 95 *d*	
3′		75 18 *d*	76 75 *d*	72 86 *d*	73 03 *d*	76 68 *d*	
4′		70 52 *d*	70 43 *d*	68 54 *d*	68 64 *d*	70 43 *d*	
5′		76 75 *d*	76 75 *d*	72 04 *d*	71 96 *d*	76 68 *d*	
6′		61 63 *t*	61 61 *t*	62 10 *t*	62 08 *t*	61 55 *t*	

*Additional signals from acetoxy groups

−1 99 ppm, C-5, Δδ −2 06 ppm) and of **2** with **1** (C-2″, Δδ +8 61 ppm, C-2′, Δδ −2 44 ppm) The sign and the value of these shift changes are in agreement with the general rule for carbohydrates and *O*-glycosides by which the glycosidation of a hydroxyl group causes a downfield shift (8–10 ppm) of the resonance of the α-carbon [5] and an upfield shift (1–4 ppm) of the β-carbons [6–8]

Chemical evidence supporting structure **5** is provided by the selective hydrogenolysis of both free allylic CH_2OH groups of **5** Thus the hepta-*O*-acetyl derivative **6** is converted by lithium/ammonia reduction at −60° (Birch reaction) into 3′,4′-bisdeoxyeucommioside II (**8**) the ^{1}H NMR spectrum (see Experimental) of which shows the presence of both expected vinylic methyl groups (broad singlet at δ 1 63, 6H) instead of the corresponding CH_2OH resonance of **5** (δ 4 24, 4H) Analogous features are present in the ^{13}C NMR spectrum of **8** (Table 1) in which the signals of allylic CH_2OH carbons (δ 57 83, 56 16) are replaced by those (δ 11 95 and 13 71) of the corresponding methyl groups The resonances of the remaining aglycone carbons of **8** correspond closely to those of 3′,4′-bisdeoxyeucommiol (**9**) apart from the expected glucosidation shifts (α and β effects) on C-1 (Δδ +8 40 ppm), C-2 (Δδ −2 11 ppm) and C-5 (Δδ −2 16 ppm)

The formation of **8** unequivocally proves the structure

of 1-*O*-β-D-glucopyranosyl eucommiol for eucommioside II (**5**) The autumnal presence of **1** and **5** in *A japonica*, as that of **1** and **2** in *Eucommia ulmoides* [1, 2], should not be interpreted as a casual co-occurrence of these compounds with aucubin (**3**), the most abundant iridoidic component of these plants present throughout the year, but rather as a possible, widespread degradation process of aucubin (**3**) which in a short and well defined period of the year is transformed into cyclopententetrol derivatives The partial synthesis of **1** from **4** (two steps) which we have recently carried out [unpublished work], can be considered an indirect proof of this hypothetical relationship

This hypothesis needs to be tested by analysis for the presence of these or similar cyclopentan(en) polyols in a series of plants rich in iridoid glucosides, mainly aucubin As a first approach to this research a careful re-examination of *Eucommia ulmoides* collected in autumn is in progress

EXPERIMENTAL

CC silica gel 70–230 mesh, TLC silica gel 60 F_{254} (Merck), PC Schleicher & Schull No 2043 Mgl paper, Spray reagents 2 N H_2SO_4, heating at 120° (silica gel plates), vanillin (vanillin 1 g, conc HCl 2 ml, MeOH 100 ml), heating at 100° (PC) All evaporations of volatile material were performed under red pres ^{1}H NMR 60, 80 and 90 MHz, HDO as int standard at 4 70 ppm for D_2O and TMS for $CDCl_3$

Isolation of iridoid fraction A japonica (3 kg of fresh leaves and branches collected in the autumn) was roughly chopped and extracted with H_2O (2 × 10 l) at 100° for 2 hr PC of the extract (BuOH–HOAc–H_2O, 63 10 27, visualized with vanillin) showed the presence of eucommiol (**1**) (R_f 0 51, olive-brown), aucubin (**3**) (R_f 0 28, pink–lilac) and a highly polar compound (R_f 0 17, olive-brown) later identified as eucommioside II (**5**) The aq extract was evaporated *in vacuo* and transferred to a column of silica gel (1 5 kg) Elution with BuOH satd with H_2O afforded successively **1** (5 g), **3** (61 g), a mixture of **3** and **5** (69 g) and finally crude **5** (20 g)

Eucommioside II (**5**) Crude **5** (20 g) was chromatographed twice on silica gel (0 5 kg) eluting with CH_2Cl_2–MeOH–H_2O (15 10 1) to give **5** (9 g) still contaminated by sugar impurities Further chromatography on silica gel (0 3 kg) eluting with Me_2CO–H_2O (19 1) afforded pure **5** (3 g) (Found C, 50 98, H, 8 09 $C_{15}H_{28}O_9$ requires C, 51 12, H, 8 01%) $[\alpha]_D^{25} = -38\,6°$ (*c* 2 8, H_2O), UV λ_{max}^{EtOH} nm (log ε) 208 (3 6)

^{1}H NMR of **5** (D_2O, 80 MHz) δ 4 55 (*d*, $J_{1\,2}$ = 7 5 Hz, anomeric H-1'), 4 40 (*m*, H-1), 4 24 (*br s*, 2H-3', 2H-4'), 3 72 (*t*, *J* = 7 0 Hz, 2H-2''), 2 88 and 2 50 (*br dd*, *br d*, J_{AB} = 18 0 Hz, 2H-5), 3 00 (*br d*, H-2), 2 3–1 2 (*cm*, 2H-2') ^{1}H NMR of **2** (D_2O, 90 MHz) [2] δ 4 50 (*d*, $J_{1\,2}$ = 7 5 Hz, anomeric H-1'), 4 24 (*br s*, H-1, 2H-3', 2H-4'), 3 9–3 6 (2H-2''), 2 82 (*m*, H-2), 2 90 and 2 32 (*br dd*, *br d*, J_{AB} = 18 0 Hz, 2H-5), 2 2–1 2 (*cm*, 2H-2') ^{1}H NMR of **1** (D_2O, 90 MHz) [1] δ 4 24 (*br s*, H-1, 2H-3', 2H-4'), 3 71 (*t*, *J* = 7 0 Hz, 2H-2''), 2 72 (*m*, H-2), 2 90 and 2 32 (*br dd*, *br d*, J_{AB} = 18 0 Hz, 2H-5), 2 1–1 2 (*cm*, 2H-2')

Enzymatic hydrolysis of **5** Eucommioside II (**5**, 90 mg) was completely hydrolysed in 2 hr at 25° with β-glucosidase (EC 3 2 1 21, Fluka, 20 mg) in H_2O (3 ml) The aq soln was extracted with EtOAc (10 ml, × 7) and the residue of the organic phase (48 mg) was chromatographed on silica gel (5 g) Elution with $CHCl_3$–MeOH (9 1) afforded the pure aglycone (34 mg) whose physical data were identical with those of an authentic sample of **1**

Hepta-O-acetyleucommioside II (**6**) Compound **5** (0 2 g) was dissolved in dry C_5H_5N (1 5 ml) and Ac_2O (3 ml) and allowed to stand at room temp for 1 hr whereupon it was added to MeOH (4 ml) at 0° After 30 min, the soln was concd under red pres and Et_2O was added to the residue The Et_2O soln was washed with cold aq HCl, H_2O and dried (Na_2SO_4) The solvent was evaporated and the residue (260 mg) chromatographed on silica gel (20 g) Elution with CH_2Cl_2–Et_2O (4 1) gave **6** (220 mg) as a colourless viscous oil ^{1}H NMR of **6** ($CDCl_3$, 60 MHz) δ 4 70 (*br s*, 2H-3', 2H-4'), 4 60 (*br s*, overlapped, H-1), 4 3–4 0 (2H-2''), 3 10 and 2 35 (*br dd*, *br d*, J_{AB} = 18 0 Hz, 2H-5), 2 68 (*br d*, *J* = 5 0 Hz, H-2), 2 3–1 3 (*mc*, 2H-2')

3',4'-Bisdeoxyeucommioside II (**8**) Liquid NH_3 (100 ml) was added to the hepta-acetate **6** (0 2 g) dissolved in EtOH (5 ml), and then, with stirring, Li (0 3 g) was added in small pieces over a period of 2 hr, keeping the temp at −60° The blue final soln was decolourized with EtOH (10 ml) and left overnight to allow the NH_3 to evaporate The final suspension was neutralized by bubbling with CO_2, centrifuged and the residue washed with EtOH (10 ml, × 10) The collected solns were concd under red pres, to give a residue (0 4 g) which when chromatographed on silica gel gave pure **8** (55 mg) as a viscous, colourless oil ^{1}H NMR of **8** (D_2O, 80 MHz) δ 4 51 (*d*, $J_{1\,2}$ = 7 5 Hz, anomeric H-1'), 4 27 (*se*, H-1), 3 67 (*t*, *J* = 7 0 Hz, 2H-2''), 2 56 (overlapped, H-2), 2 73 and 2 25 (*br dd*, *br d*, J_{AB} = 18 0 Hz, 2H-5), 2 1–1 2 (*cm*, 2H-2'), 1 63 (*br s*, 3H-3', 3H-4')

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